Exporing the vitellogenin gene in vivo

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Summary

The regulation of expression of the chicken vitellogenin II (Vtg) gene, which encodes a yolk precursor protein, is the main subject of this thesis. The Vtg gene is expressed in the liver only. Transcriptional activation of the gene is fully dependent on the presence of estrogen. Transcription normally occurs in laying hen, but it can also be induced in immature chickens and roosters by administration of estradiol. This allows us to investigate induction of gene expression in a highly specialized, fully differentiated organ. In Chapter 1, a brief review of eukaryotic gene expression is given. Chapter 2 describes the relationship between hormonal status and DNA methylation of the Vtg gene region in different organs. The transcribed region of the gene is gradually demethylated upon prolonged transcription in the liver of laying hen. Probably, this demethylation does not play a major part in regulation of the gene. During this work, we encountered a polymorphism in the 3' part of the Vtg gene. The region differing from the previously isolated allele was cloned. It appeared to contain an insertion of about 600 bp, which consisted of two partial duplications of introns 32 and 33 placed in tandem in intron 33. A model proposing a possible mechanism by which these duplications have arisen, is presented (Chapter 3). Chapters 4 and 5 deal with protein-DNA interactions in the 5' flanking region of the Vtg gene. Using dimethyl sulphate (DMS) and DNaseI footprinting, we show that control elements become occupied *in vivo* by proteins after induction of the gene. Among the protein binding sites revealed are the estrogen responsive elements, the probable target sites of the estradiol-receptor complex, and a sequence resembling the binding site of nuclear factor 1. The interaction of nuclear proteins with this site has been further characterized by binding experiments *in vitro* (Chapter 5, part 1). The interesting observation was made that the estradiol-receptor complex and the nuclear factor 1 like protein bind to their target sequences in front of the Vtg gene in the expressing liver only, and not for example in the oviduct, although they are also present in this organ. The tissue-specificity of Vtg gene expression must therefore reside in other DNA regions and/or different proteins. The other protein binding sites described are obvious candidates.

They are generally located in elements conserved between yolk protein genes, but the proteins interacting with them have not been identified yet. They may now be characterized *in vitro*. It is conceivable that the molecular events governing estrogen-controlled gene expression will be elucidated within the next decade.